12/22/2022

2022.12.22 Project Skunkworks: Just Primers™ Synthesis Concept

1. (B)~~biotin~~ 5′-GCTACCGGtagcttctagtggtacgccgcatacgtcatacc - 3′
   ||||||||
   3′-CGATGGCC-dT-5′

- **Under Subheading 1:** an oligonucleotide tethered to a solid substrate **(B)** like a magnetic bead or polystyrene bead via a biotin link. The sequence labeled in red represents the pioneering oligo on which *de novo* enzymatic synthesis is conducted. The tethered ssDNA sequence is the reverse complement of a primer sequence of interest. The bead proximal oligo hybridized to the tethered ssDNA strand contains an inverted T nucleotide that prevents a covalent link from forming with an upstream nucleotide.

2. (B)~~biotin~~ 5′-GCTACCGGtagcttctagtggtacgccgcatacgtcatacc - 3′
   |||||||| ||||||||||||||||||||||||||||
   3′-CGATGGCC-dt aagatgaccatgcggcgtatgcagtatgg - 5′

- **Under Subheading 2:** Sea Vent Polymerase™ is a primer independent polymerase that fills in the anti-sense strand. The primer proximal oligo can or not be crosslinked by EMA treatment before oligo synthesis.

3. (B)~~biotin~~ 5′-GCTACCGGtagcttctagtggtacgccgcatacgtcatacc - 3

   3′-CGATGGCC-dT -5′ + 3′ - aagatgaccatgcggcgtatgcagtatgg - 5′

- **Under Subheading 3: 1M NaOH melts the non-tethered anti-sense strand. The inverted T on the bead proximal oligo on the antisense strand prevents extension of the “primer” molecule into the tethering oligo. The non tethered products are collected.**

4. (B)~~biotin~~ 5′-GCTACCGGtagcttctagtggtacgccgcatacgtcatacc - 3′
   ||||||||
   3′-CGATGGCC-dT-5′

- **After melting off the extended strand, the original tethered ssDNA can be reused to generate more oligo.**

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